CLAIMS

What is claimed is:

5   1.   A method of determining the specific residues binding to a target of interest within a known parent polypeptide that binds to the target of interest, comprising the steps of:

      (a)   providing a known parent polypeptide that binds to a target of interest with a known primary structure, such primary structure consisting of n residues;

      (b)   constructing a first peptide of the formula R_1-Z-R_2,

10   wherein

      R_1 comprises from 2 to n residues, such residues the same as or homologs of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure;

      Z is a residue or mimetic thereof providing both a nitrogen atom (N) and a sulfur atom (S) for metal ion complexation;

15   R_2 comprises from 0 to n - 2 residues, such residues the same as or homologs of residues in the parent polypeptide and in the same order as residues in the parent polypeptide primary structure, and forming with R_1 a sequence in the same order as in the parent polypeptide primary structure with Z either inserted between two adjacent residues corresponding to two adjacent residues in such primary structure or substituting for a single residue corresponding to a single residue in such

20   primary structure, and wherein the residues comprising R_1-Z-R_2 are equal to either n or n +1;

      (c)   complexing the first peptide of the formula R_1-Z-R_2 to a metal ion, thereby forming a first R_1-Z-R_2 metallopeptide;

      (d)   screening the first R_1-Z-R_2 metallopeptide for binding to the target of interest;

      (e)   repeating steps (b) through (d), wherein the resulting R_1-Z-R_2 metallopeptide

25   differs in at least either R_1 or R_2; and

      (f)   selecting the R_1-Z-R_2 metallopeptide exhibiting substantially decreased binding to the target of interest, whereby at least one residue of the sequence binding to the metal ion of such R_1-Z-

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R_2 metallopeptide comprises the identification of the specific residues of the parent polypeptide binding to the target of interest.

2. The method of claim 1 wherein Z is an L- or D-3-mercapto amino acid.

3. The method of claim 2 wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homolog of any of the foregoing.

4. The method of claim 1 wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Re, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

5. The method of claim 1 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

6. The method of claim 1 wherein screening for binding to the target of interest comprises competing a known binding partner for binding to the target of interest with the R_1-Z-R_2 metallopeptide.

7. The method of claim 6 wherein the known binding partner is the parent polypeptide.

8. The method of claim 1 wherein screening for binding to the target of interest comprises a functional assay.

9. The method of claim 1 wherein the target of interest is a biological receptor capable of transmitting a signal, and screening further comprises determining whether the R_1-Z-R_2 metallopeptide induces decreased transmission of the signal.

10.   A method of determining the specific residues binding to a target of interest within a known primary sequence parent polypeptide that binds to the target of interest, comprising the steps of:

(a)   making a series of peptides, wherein each peptide in the series includes the known primary sequence of the parent polypeptide and a single inserted L- or D-3-mercapto amino acid residue, with the single L- or D-3-mercapto amino acid inserted for each peptide at each position along the primary sequence from the position between the second and third residues from the N-terminus through the C-terminus position;

(b)   complexing each peptide in the series with a metal ion to form a series of metallopeptides; and,

(c)   determining the binding of each metallopeptide of the series of metallopeptides to the target of interest.

11.   The method of claim 10, further comprising the step of selecting the metallopeptide or metallopeptides of the series exhibiting substantially decreased binding to the target of interest.

12.   The method of claim 10, wherein any L- or D-3-mercapto amino acid residue in the series of peptides other than the single inserted L- or D-3-mercapto amino acid residue further comprises a sulfur protecting group, whereby the sulfur therein cannot complex a metal ion.

13.   The method of claim 10, wherein any L- or D-3-mercapto amino acid residue in the series of peptides other than the single inserted L- or D-3-mercapto amino acid residue is substituted with a homolog.

14.   The method of claim 10, wherein for any peptide in the series containing a proline residue as either of the two residues on the immediately adjacent N-terminus side of the single inserted L- or D-3-mercapto amino acid residue, the proline residue is substituted with a homolog.

15. The method of claim 10, wherein the L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homolog of any of the foregoing.

5   16. The method of claim 10, wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Re, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

17. The method of claim 16, wherein the metal ion is an ion of technetium or rhenium.

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18. The method of claim 10 wherein the target of interest is a receptor, antibody, toxin, enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular protein domain of biological relevance.

15   19. The method of claim 10, wherein determining the binding of each metallopeptide of the series of metallopeptides to the target of interest comprises competing a known binding partner for binding to the target of interest with each metallopeptide.

20. The method of claim 10, wherein determining the binding of each metallopeptide of the

20   series of metallopeptides to the target of interest comprises a functional assay.

21. The method of claim 10, wherein the target of interest is a biological receptor capable of transmitting a signal, and wherein determining the binding of each metallopeptide of the series of metallopeptides to the target of interest comprises determining whether each metallopeptide induces

25   decreased transmission of the signal.

22   A method of determining the specific residues binding to a target of interest within a
known primary sequence parent polypeptide that binds to the target of interest, comprising the steps of:

(a)   making a series of peptides, wherein each peptide in the series includes the
known primary sequence of the parent polypeptide with a single substitution, the single substituent

5   consisting of an L- or D-3-mercapto amino acid residue substituted at each position along the primary
sequence from the third residue from the N-terminus through the C-terminus residue;

(b)   complexing each peptide in the series with a metal ion to form a series of
metallopeptides; and,

(c)   determining the binding of each metallopeptide of the series of metallopeptides to

10   the target of interest.

23.   The method of claim 22, further comprising the step of selecting the metallopeptide or
metallopeptides of the series exhibiting substantially decreased binding to the target of interest.

15   24.   The method of claim 22, wherein any L- or D-3-mercapto amino acid residue in the series
of peptides other than the single substituent L- or D-3-mercapto amino acid residue further comprises a
sulfur protecting group, whereby the sulfur therein cannot complex a metal ion.

25.   The method of claim 22, wherein any L- or D-3-mercapto amino acid residue in the series

20   of peptides other than the single substituent L- or D-3-mercapto amino acid residue is substituted with a
homolog.

26.   The method of claim 22, wherein for any peptide in the series containing a proline residue
as either of the two residues on the immediately adjacent N-terminus side of the single substituent L- or

25   D-3-mercapto amino acid residue, the proline residue is substituted with a homolog.

27.   The method of claim 22, wherein the L- or D-3-mercapto amino acid is L- or D-cysteine,

L- or D-penicillamine, 3-mercapto phenylalanine, or a homolog of any of the foregoing.

28.   The method of claim 22, wherein the metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga,

5   As, Se, Y, Mo, Tc, Ru, Rh, Re, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or

Gd.

29.   The method of claim 28, wherein the metal ion is an ion of technetium or rhenium.

10   30.   The method of claim 22, wherein the target of interest is a receptor, antibody, toxin,

enzyme, hormone, nucleic acid, intracellular protein domain of biological relevance or extracellular

protein domain of biological relevance.

31.   The method of claim 22, wherein determining the binding of each metallopeptide of the

15   series of metallopeptides to the target of interest comprises competing a known binding partner for

binding to the target of interest with each metallopeptide.

32.   The method of claim 22, wherein determining the binding of each metallopeptide of the

series of metallopeptides to the target of interest comprises a functional assay.

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33.   The method of claim 22, wherein the target of interest is a biological receptor capable of

transmitting a signal, and wherein determining the binding of each metallopeptide of the series of

metallopeptides to the target of interest comprises determining whether each metallopeptide induces

decreased transmission of the signal.

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34. A metallopeptide library for determining the specific residues binding to a target of interest within a known primary sequence parent polypeptide of at least five amino acid residues that binds to the target of interest, comprising:

a series of metellapeptides, wherein each metallopeptide within the series includes the

5   known primary sequence of the parent polypeptide and a single inserted L- or D-3-mercapto amino acid residue, with the single L- or D-3-mercapto amino acid inserted for each peptide at each position along the primary sequence from the position between the second and third residues from the N-terminus through the C-terminus position, and a metal ion complexed to the sequence comprising the single inserted L- or D-3-mercapto amino acid and the two residues on the immediately adjacent N-terminus

10   side of the single inserted L- or D-3-mercapto amino acid residue,

wherein any L- or D-3-mercapto amino acid residue in the series of peptides other than the single inserted L- or D-3-mercapto amino acid residue either further comprises a sulfur protecting group, whereby the sulfur therein cannot complex a metal ion, or a homolog, and further wherein for an metallopeptide in the series containing a proline residue as either of the two residues on the immediately

15   adjacent N-terminus side of the single inserted L- or D-3-mercapto amino acid residue, the proline residue is substituted with a homolog.

35. The metallopeptide library of claim 34, wherein the inserted L- or D-3-mercapto amino acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homolog of any of the

20   foregoing.

36. The metallopeptide library of claim 34, wherein metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Re, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po, At, Sm, Eu or Gd.

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37. The metallopeptide library of claim 36, wherein the metal ion is an ion of technetium or rhenium.

38.   A metallopeptide library for determining the specific residues binding to a target of interest within a known primary sequence parent polypeptide of at least five amino acid residues that binds to the target of interest, comprising:

5   a series of metellapeptides, wherein each metallopeptide within the series includes the known primary sequence of the parent polypeptide with a single substitution, the single substituent consisting of an L- or D-3-mercapto amino acid residue substituted at each position along the primary sequence from the third residue from the N-terminus through the C-terminus residue, and a metal ion complexed to the sequence comprising the single substituent L- or D-3-mercapto amino acid and the two

10   residues on the immediately adjacent N-terminus side of the single substituent L- or D-3-mercapto amino acid residue,

                wherein any L- or D-3-mercapto amino acid residue in the series of peptides other than the single substituent L- or D-3-mercapto amino acid residue either further comprises a sulfur protecting group, whereby the sulfur therein cannot complex a metal ion, or a homolog, and further wherein for an

15   metallopeptide in the series containing a proline residue as either of the two residues on the immediately adjacent N-terminus side of the single substituent L- or D-3-mercapto amino acid residue, the proline residue is substituted with a homolog.

        39.   The metallopeptide library of claim 38, wherein the substituent L- or D-3-mercapto amino

20   acid is L- or D-cysteine, L- or D-penicillamine, 3-mercapto phenylalanine, or a homolog of any of the foregoing.

        40.   The metallopeptide library of claim 38, wherein metal ion is an ion of V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Y, Mo, Tc, Ru, Rh, Re, Pd, Ag, Cd, In, Sn, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Po,

25   At, Sm, Eu or Gd.

41.   The metallopeptide library of claim 40, wherein the metal ion is an ion of technetium or rhenium.